

γ -Radiation Influences Browning, Antioxidant Activity, and Malondialdehyde Level of Apple Juice

XUETONG FAN* AND DONALD W. THAYER

Food Safety Research Laboratory, Eastern Regional Research Center, Agricultural Research Service,
U.S. Department of Agriculture, 600 East Mermaid Lane, Wyndmoor, Pennsylvania 19038

Apple juice was γ -irradiated at 5 °C at doses ranging from 0 to 8.9 kGy and then stored at 5 °C for 15 days. Ionizing radiation reduced the browning of apple juice and increased antioxidant activity measured by the ferric-reducing antioxidant power (FRAP) assay. The magnitude of changes increased with radiation dose. The level of malondialdehyde (MDA) measured using the thiobarbituric acid reactive substrates assay increased at radiation doses above 2.67 kGy. The browning of irradiated juices increased during storage at 5 °C, but the irradiated juices were still lighter than controls at the end of storage. Differences in FRAP values disappeared during early periods of storage while higher MDA levels were observed in irradiated samples during most of the storage period. Elimination of suspended matter from apple juice did not alter irradiation-induced changes in browning, FRAP, or MDA formation. As compared to irradiation conducted at 5 and 20 °C, treatment at –15 °C was less effective in reducing browning and in increasing MDA formation but elevated FRAP values. The exclusion of oxygen from juices did not affect the reduction in browning due to irradiation but promoted the increase in FRAP values and decreased the irradiation-induced MDA formation.

KEYWORDS: Irradiation; antioxidant; browning; malondialdehyde; temperature; oxygen

INTRODUCTION

Outbreaks of several human diseases have been linked with the consumption of unpasteurized fruit juices contaminated with food-borne pathogens (1). Although thermal pasteurization can eliminate pathogens, the process may impair the characteristic flavor of juices (2). Several nonthermal techniques have been shown to be capable of achieving the 5-log reduction of common human pathogens (3). One of these techniques is ionizing irradiation, which not only inactivates food-borne pathogens but also reduces spoilage and degrades mycotoxins (4–7). The effect of irradiation on the quality of apple juice/cider is, however, a concern. Earlier studies have demonstrated that irradiation at doses less than 20 kGy did not affect sugar content and acidity of juices (7–9). The impact of irradiation on other quality attributes is unclear.

The objective of most of the earlier studies was to achieve a stable product at ambient temperature (20–25 °C); therefore, sterilizing radiation doses (> 10 kGy) were employed. Depending on the species and strains of pathogens, doses of 1–3.55 kGy are sufficient to achieve the 5-log reduction of common pathogens (4, 10) recommended by the National Advisory Committee on Microbiological Criteria for Food (11).

Fruits and fruit juices contain large amounts of antioxidants. Recent studies have highlighted the importance of antioxidants

for protection from heart disease and cancers (12). Many fruit juices contain considerable amounts of ascorbic acid, which can be partially destroyed by irradiation (6, 9, 13). The decomposition of ascorbic acid can be prevented by irradiating juices in the frozen state or under nitrogen (14). Unfortified apple juice, however, does not contain a significant amount of ascorbic acid, but it does have many other antioxidants, such as phenolics (15). The loss of these antioxidants due to irradiation treatment in apple juice is unknown.

In addition to losses of ascorbic acid, irradiation can also affect color. Some earlier studies on irradiation-induced changes in juice color are controversial. Chachin and Ogata (8) reported that irradiated apple juice became brown. Kiss and Farkas (5) showed that concentrated apple juice became brighter after irradiation at 10 kGy. After storage at 50 or 60 °C, it redarkened. The magnitude of the impact caused by irradiation at the doses of pathogen inactivation is unclear.

It has been reported that aqueous solutions of carbohydrates irradiated at 25 kGy inhibit the growth of mammalian cells in culture (16), and it has been postulated that malondialdehyde (MDA) together with other carbonyl compounds may contribute to this effect (17). MDA is mutagenic to *Salmonella* tester strains (18, 19) and initiates skin tumors in mice (20). Therefore, accumulation of MDA in juices is of concern. It has been shown that high doses of irradiation induce MDA formation in aqueous carbohydrate solutions (21, 22). The accumulation of MDA in juices irradiated at relatively low doses, i.e., 1–3.55 kGy, has not been studied.

* To whom correspondence should be addressed. Tel: (215)836-3785. Fax: (215)233-6406. E-mail: xfan@arserrc.gov.

The objectives of this study are to investigate the effect of irradiation of apple juice at doses of pathogen inactivation on color, antioxidant activity, and MDA formation and also to explore ways of reducing adverse effects of irradiation.

MATERIALS AND METHODS

Juice Samples. Pasteurized apple juice (not from concentrates) that contained suspended matters was purchased locally. The juice was stored at 5 °C prior to use. We used pasteurized juice instead of fresh juice to eliminate any possible effect of microorganisms that may be present in fresh juice.

Effect of Irradiation Dose and Storage. Approximately 4 mL of juice was placed into 5 mL glass vials and then sealed with Teflon-lined septa and screw caps. The vials containing juice were stored at 5 °C overnight and then irradiated with 0, 0.89, 1.78, 2.67, 3.56, 4.55, and 8.90 kGy γ -radiation at 5 °C. After irradiation, the juice was stored at 5 °C for 15 days. Browning, antioxidants, and MDA were measured immediately after irradiation and after 2, 4, 7, 11, and 15 days of storage. Juices were centrifuged at 1300g for 10 min in a Sorvall RT6000B centrifuge (DuPont Co., Wilmington, DE) at 23 °C to remove suspended solids, and the supernatant was used for analysis. There were four replicates for each treatment. Each vial was regarded as a replicate.

Effect of Suspended Matter on the Irradiation Effect. Juice was filtered through a 0.45 μ m membrane (Type HV, Millipore Co., Bedford, MA) under vacuum. Then, both filtered and nonfiltered juices in 5 mL glass vials were irradiated at 5 °C at the above doses. Browning, antioxidants, and MDA were assayed on the day of irradiation. Nonfiltered irradiated juice was filtered through a 0.45 μ m membrane before analysis.

Effect of Processing Temperature. Juice (4 mL) was placed in 5 mL vials, sealed with Teflon-lined septa and caps, and stored at -15, 5, and 20 °C overnight before irradiation at 9.4 kGy at the corresponding temperatures. The samples were then placed at 5 °C for approximately 6 h to allow the frozen sample to thaw. The juice was centrifuged at 1300g for 10 min, and the supernatant was used for analysis.

Effect of Oxygen Exclusion. Approximately 3.5 mL of juice was placed into 5 mL vials and then sealed with Teflon-lined septa and open-top screw caps. Two hypodermic needles (26 gauge) were inserted through the septum to serve as gas ports. Oxygen in the juice and headspace of the vials was stripped by flushing the juice with nitrogen for 2 min through the needles at a flow rate of 50 mL min⁻¹. The juice was then irradiated at 9.4 kGy at 20 °C. Our results indicated that the nitrogen in the vials was retained for at least 12 h.

Irradiation and Dosimetry. Irradiation was conducted using a self-contained, Lockheed Corporation ¹³⁷Cs γ -radiation source (Marietta, GA). The unit has 23 ¹³⁷Cs pencils placed in an annular array around a 63.5 cm high stainless steel cylindrical chamber with a 22.9 cm internal diameter. The source strength at the time of this study was ca. 109 000 Ci (4.0 PBq) with a dose rate of 0.10 kGy min⁻¹. The dose rate was established using alanine transfer dosimeters from the National Institutes of Standards and Technology (Gaithersburg, MD). Corrections for source decay were made monthly. Variations in radiation dose absorption were minimized by placing the samples within a uniform area of the radiation field and by irradiating them within a polypropylene container (4 mm wall) to absorb Compton electrons and by using the same geometry for sample irradiation during the entire study. Routine dosimetry was performed using 5 mm diameter alanine dosimeters (Bruker Instruments, Rjeomstettem, Germany), and the free-radical signal was measured using a Bruker EMS 104 EPR Analyzer (23). The dosimeters were placed into 1.2 mL cryogenic vials (Nalgene, Rochester, NY), and the cryogenic vials were taped onto the tubes containing juice samples prior to irradiation. The temperature in the radiation chamber was maintained by injecting the gas phase from a liquid nitrogen tank into the radiation chamber.

Antioxidant Determination. Antioxidant activity was determined using the ferric-reducing antioxidant power (FRAP) assay (24, 25) by the addition of 3 mL of FRAP reagent to 100 μ L of supernatant. The FRAP reagent was prepared daily by combining 300 mM acetate buffer (pH 3.6), 10 mM 2,4,5-tripyrilidyl-s-triazine in 40 mM HCL, and 20 mM FeCl₃ in the ratio of 10:1:1 (v:v:v). The mixture was incubated at

23 °C for 30 min, and then, absorbance at 593 nm was measured with a Sargent-Welch 6-550 UV/VIS spectrophotometer (Pye Unicam Ltd., Cambridge, U.K.). FRAP values were calculated from FeSO₄ standard curves (24).

Browning Assay. The absorbance spectrum of juice was measured using a Shimadzu UV-1601 spectrophotometer (Shimadzu Scientific Instruments, Columbia, MD). Quartz cuvettes (Thomas Scientific, Swedesboro, NJ) suitable for 220–4000 nm were used. For the routine measurement of browning, the absorbance at 420 nm was measured according to Meydav and others (26) using the Sargent-Welch 6-550 UV/VIS spectrophotometer (Pye Unicam Ltd., Cambridge, U.K.).

MDA Measurement. MDA was measured using the thiobarbituric acid reactive substrates (TBARS) assay (27). A 1.6 mL sample of 4 times the diluted juice was added to a test tube containing 1.6 mL of either (i) –thiobarbituric acid (TBA) solution, 20% (w/v) trichloroacetic acid, and 0.01% butylated hydroxytoluene or (ii) +TBA solution, containing the above plus 0.65% TBA. Samples were then mixed vigorously, heated at 95 °C in a water bath for 25 min, cooled, and centrifuged at 1300g for 10 min at 5 °C. The absorbance at 440, 532, and 600 nm was monitored using a Shimadzu UV-1601 spectrophotometer (Shimadzu Scientific Instruments, Columbia, MD). MDA was calculated using the formulas developed by Hodges et al. (27).

$$\frac{[(\text{Abs}_{532+\text{TBA}} - \text{Abs}_{600+\text{TBA}}) - (\text{Abs}_{532-\text{TBA}} - \text{Abs}_{600-\text{TBA}})]}{0.0571} = A \quad (1)$$

$$[(\text{Abs}_{440+\text{TBA}} - \text{Abs}_{600+\text{TBA}})0.0571] = B \quad (2)$$

$$\text{MDA (nmol mL}^{-1}\text{)} = [(A - B)/157\,000] 10^6 \quad (3)$$

Statistical Analysis. The effect of radiation dose was analyzed using linear and nonlinear regression, and mean separation was achieved by the least significant difference (LSD) analysis of GLM procedure using SAS version 7 (SAS Institute, Raleigh, NC). In some of the figures, mean standard deviations are presented. Differences between means that exceed the standard deviations were always significant when analyzed using the LSD procedure at the $P < 0.05$ level.

RESULTS

Dose and Storage Effect. Irradiation decreased juice browning as measured using absorbance at 420 nm (**Figure 1**). At 0.89 kGy, $A_{420\text{nm}}$ was reduced by 60.5%. The effect of irradiation on browning was exponential. As the dose increased, the decrease in $A_{420\text{nm}}$ slowed. The absorbance spectra of juice indicated that the reduction of absorbance was extended from visible range to UV range (**Figure 2**).

FRAP values increased linearly as doses were raised from 0.89 to 8.9 kGy (**Figure 1**). At 0.89 kGy, irradiation did not have any significant influence ($P < 0.05$) on FRAP values (**Figure 1**). MDA formation increased ($P < 0.05$) at radiation doses of 2.67 kGy and above.

During storage at 5 °C, $A_{420\text{nm}}$ of nonirradiated juice did not change while irradiated juice increased during storage, indicating that irradiated juice darkened during storage (**Figure 3**). The lightness of irradiated juice was still evident after 15 days of storage. Although juices irradiated at doses above 1.78 kGy had significantly ($P < 0.05$) higher FRAP values measured immediately after irradiation and 2 days after irradiation, there was no difference between those not irradiated and those irradiated at 4.45 kGy or below after 4 days of storage. Overall, MDA levels of juice irradiated at doses of 2.67 kGy and above were significantly ($P < 0.05$) higher than those in nonirradiated juice during storage at 5 °C.

Effect of Suspended Matter. As compared to nonfiltered juices, juices free of suspended matter had small but significantly ($P < 0.05$) less browning at doses of 2.67 and 4.45 kGy (**Figure**

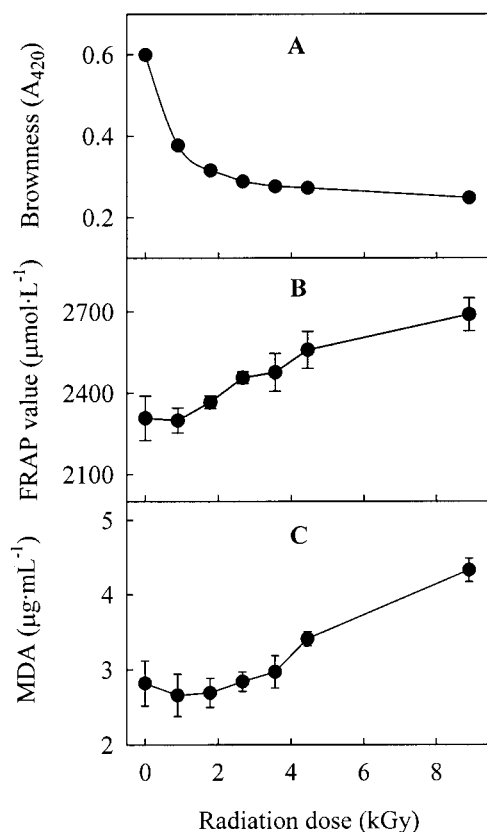


Figure 1. Effect of irradiation on browning intensity (A), FRAP values (B), and MDA level (C) of apple juice. Juice was irradiated at doses of 0, 0.89, 1.78, 2.67, 3.56, 4.45, and 8.90 kGy at 5 °C. Vertical bars represent standard deviations of means. The absence of vertical bars indicates that the standard deviation was smaller than the symbol.

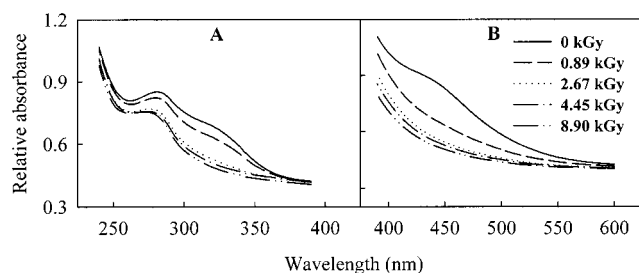


Figure 2. UV (A) and visible (B) absorption spectra of nonirradiated and irradiated apple juice measured on the same day of irradiation. Juice was irradiated at doses of 0, 0.89, 2.67, 4.48, and 8.90 kGy at 5 °C. For the measurement of absorbance at the UV range, the juice was diluted 4 times with deionized water.

4A). Overall, suspended matter had little effect on irradiation-induced changes in browning, FRAP values, and MDA level in the dose range of 0–8.9 kGy (Figure 4).

Effect of Processing Temperature. The reduction of browning by irradiation was less effective when juices were irradiated frozen (−15 °C) as compared to 5 and 20 °C (Table 1). There was no difference on the bleaching effect of irradiation between 5 and 20 °C. The increase in FRAP values by irradiation was more effective at −15 °C while MDA formation increased with raised processing temperature.

Effect of Oxygen Exclusion (Nitrogen Flushing). As compared to those without nitrogen flushing, flushing with nitrogen prior to irradiation increased the FRAP values and decreased the MDA formation and had no effect on juice browning (Table 2).

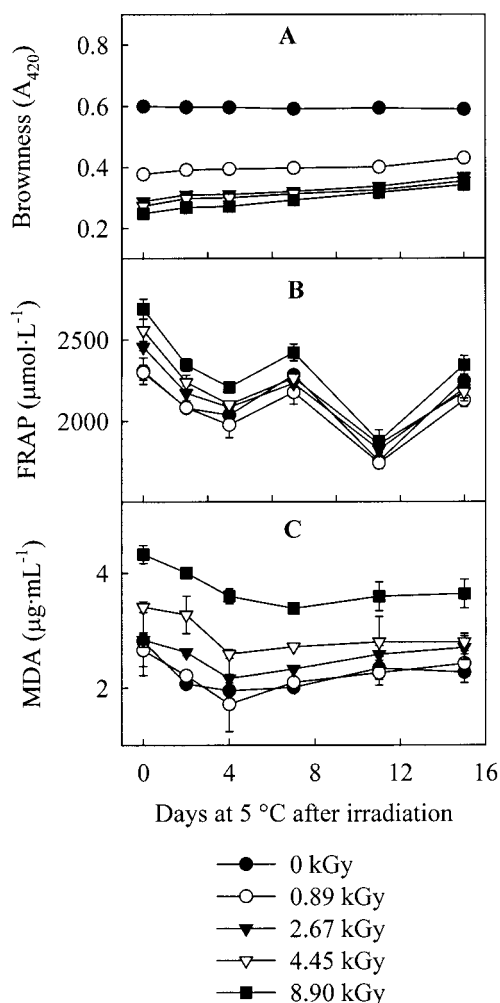


Figure 3. Changes in browning intensity (A), FRAP values (B), and MDA level (C) of nonirradiated and irradiated apple juice during storage at 5 °C. Juice was irradiated at doses of 0, 0.89, 1.78, 2.67, 3.56, 4.45, and 8.90 kGy at 5 °C. Only data from 0, 0.89, 2.67, 4.45, and 8.9 kGy were shown for clarity. Vertical bars represent standard deviations of means. The absence of vertical bars indicates that the standard deviation was smaller than the symbol.

DISCUSSION

Brown pigment formation is undesirable in many fruit juices. Our results indicate that irradiation reduced the browning of apple juice and are in agreement with an earlier report on concentrated apple juice (5). The bleaching effect of irradiation has been observed in strawberry juice (28). The effect was likely due to the formation of free radicals (29). Although irradiated apple juice darkened during storage, it was still much lighter than the controls after 15 days of storage at 5 °C (Figure 3).

Apple juice does not contain measurable amounts of carotenoids (30, 31). The light brown color of nonirradiated juice is probably due to compounds from nonenzymatic and enzymatic browning reactions that occurred during processing and storage. Among the most commonly used indicators of nonenzymatic browning products are $A_{280\text{nm}}$ for pyrazine compounds and other heterocyclics and $A_{420\text{nm}}$ for brown pigment detection (32). Maximum absorbances were observed around 280 and 420 nm in apple juice used in the present study, indicating that the brown pigments in the apple juice may be formed from nonenzymatic reactions. Patulin, a common mycotoxin in apple juice, has a maximum absorbance at 275 nm, and patulin is known to be degraded by low-dose radiation

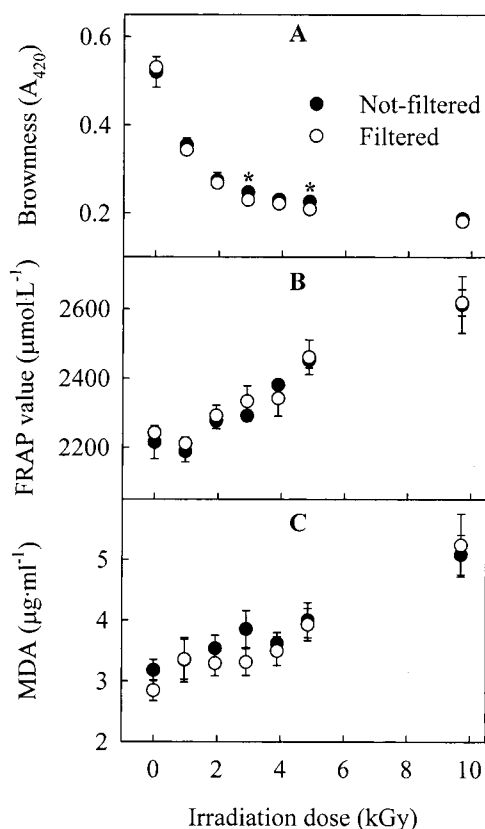


Figure 4. Effect of suspended matters on the irradiation-induced changes in browning (A), FRAP values (B), and MDA level (C) of apple juice. Juice was irradiated at doses of 0, 0.89, 1.78, 2.67, 3.56, 4.45, and 8.90 kGy at 5 °C. * indicates the significant differences analyzed using the LSD procedure at the $P < 0.05$ level.

Table 1. Processing Temperature Effect on Browning ($A_{420\text{nm}}$), FRAP Values, and MDA Level of Apple Juice^a

processing temp (°C)	$A_{420\text{nm}}$ (% of control)	FRAP values (% of control)	MDA (% of control)
-15	82.3a ^b	120.9a	108.9a
5	62.8b	109.4b	147.1b
20	61.9b	110.7b	250.6c

^a Apple juice was irradiated with γ -rays to a dose of 9.4 kGy at -15, 5, and 20 °C. ^b Means with same letters are not significantly different (LSD, $P < 0.05$).

Table 2. Effect of Nitrogen Flushing on the Irradiation-Induced Changes in Browning, FRAP Values, and MDA Level^a

flushing with nitrogen	$A_{420\text{nm}}$ (% of control)	FRAP values (% of control)	MDA (% of control)
no	61.9a ^b	110.7a	250.6a
yes	62.8a	118.6b	223.8b

^a Juice in vials flushed with or without nitrogen was irradiated with γ -rays to a dose of 9.4 kGy at 20 °C. ^b Means with same letters are not significantly different (LSD, $P < 0.05$).

(7). The observed decrease in the absorbance of irradiated juice over a broad range of wavelengths indicates that many pigments may be affected by irradiation. The specific pigments bleached by irradiation are unknown.

FRAP values as indicators of antioxidant power increased at doses above 0.89 kGy, but the high FRAP disappeared during storage at 5 °C. Apple juice contains a very low amount of ascorbic acid. The amount of ascorbic acid in the juice used in

this study contributed less than 3% of the total antioxidant power (data not shown). Major antioxidants in apple juice are phenolics (15, 30), and phenolics, such as chlorogenic acid, are much more resistant to irradiation than is ascorbic acid (data not shown). During irradiation, reductants (33) and novel new antioxidants may be formed (34). The redox state of ions and compounds may also be changed by irradiation. For example, the hydrated electrons (e_{aqueous}^-) formed from water radiolysis can react strongly with metal ions (such as Fe^{3+}) and reduce them to lower redox states (Fe^{2+}). The measurement of FRAP values is based on compounds' ability to reduce Fe^{3+} to Fe^{2+} . Therefore, the increase in FRAP values may be due to the change in the redox state of metal ions, formation of reductants, and/or formation of new antioxidants. The exact compound(s) that contributes to FRAP values remains to be elucidated. The disappearance of elevated FRAP values during storage suggests that the compounds are not stable.

The TBARS values have been used as a means of MDA measurement and widely applied as an index of lipid oxidation of meats. TBA reacts with MDA but also interacts with many other compounds (35). Although the MDA method used in this experiment had been improved and was recently developed for carbohydrate-rich foods (27), the method was not specific. Using similar methods, it has been shown that irradiation increased MDA formation of many aqueous and solid carbohydrates (21–22, 36). Our results indicate that irradiation may increase MDA formation in apple juice. However, a more specific method of MDA measurement is needed to accurately assess the effect of irradiation on MDA formation in apple juice.

The irradiation-induced changes in browning and MDA levels were influenced by the processing temperature. Irradiation was less effective in bleaching but more effective in elevating the FRAP values when juices were irradiated frozen. MDA formation increased with the processing temperature. The impact of ionizing irradiation on the chemical changes of various foods has two mechanisms, indirect and direct (37). The primary mechanism in aqueous solutions such as juice in the nonfrozen state would be the indirect effect, in which water radicals (such as e_{aqueous}^- , $\text{H}\cdot$ and $\cdot\text{OH}$) generated from the radiolysis of water react with solutes and induce changes in the chemical properties of solutions. Another mechanism of irradiation is the direct interaction of radiation with solutes, which does not involve the free radicals from water. Temperature has a great influence on the indirect effect and little effect on the direct effect. Under conditions where juice is not frozen, the chemical changes caused by irradiation will be primarily due to the reaction of the free radicals from water with juice constituents (indirect); under conditions where juice is frozen, the diffusion rate of free radicals from water and reactivity of the radical acceptors in the juice are reduced (29), and chemical changes are mostly due to the direct effect. The reduction of bleaching in frozen juice indicates that the bleaching effect observed in juice in a nonfrozen state is an indirect effect of irradiation. Radiation at -15 °C did not reduce the changes in FRAP values, indicating that the increase in FRAP values may be a result of direct interactions of juice constituents with radiation.

As compared to those irradiated in the presence of oxygen, flushing juice with nitrogen did not reduce the bleaching effect of irradiation but increased antioxidant power and reduced MDA formation. Exclusion of oxygen can neither inhibit the radiolysis of water and the subsequent attack on juice constituents nor inhibit the direct effects of radiation on juice constituents (29). The presence of oxygen only affects oxygen-related changes,

such as peroxide formation and the reaction of oxygen with free radicals. Reports on the effects of anoxia during irradiation are controversial (29). Elimination of oxygen increased the antioxidant power, indicating that oxygen is probably not involved with the formation of compounds responsible for the higher FRAP values. The increase in FRAP values in the absence of oxygen and the disappearance of the elevated FRAP values during storage indicate that oxygen may promote the degradation of the compounds responsible for the higher FRAP values observed in the irradiated juice. Antioxidants may react with hydrogen peroxide and oxygen, resulting in the loss of their antioxidant ability. The bleaching effect of irradiation is not involved with oxygen since elimination of oxygen did not affect the bleaching effect. Similar results have been shown with strawberry juice. Freezing of strawberry juice before irradiation improved color retention, while irradiation in a nitrogen atmosphere exerted little or no protective effect (28). MDA is believed to be a result of the oxidation of lipids and perhaps of carbohydrates. The presence of oxygen increases the oxidation process and therefore promotes the formation of MDA.

Irradiation increased the FRAP values at doses above 0.89 kGy. Whether the increases in FRAP values and MDA levels are due to the release of compounds from the suspended matters is unclear. Elimination of suspended matters from juices did not affect the irradiation-induced changes in browning, FRAP, and MDA formation (Figure 4), indicating that changes that occurred during irradiation are associated with solutes not suspended matter.

To achieve a goal of 5-log reductions of *Escherichia coli* O157:H7, doses of 1–2 kGy, depending on the strains, are required (4). For the control of *Salmonella*, doses of 1.80–3.55 kGy are needed (10). On the basis of the result of the present study, a dose of 2 kGy would significantly reduce browning and probably increase FRAP values but would not significantly change MDA formation on the day of irradiation. After 15 days of storage, the only benefit of irradiation would be the reduction of browning. At 3.55 kGy, an elevated level of MDA might be encountered in the irradiated juice during storage. However, the formation of MDA can be reduced by flushing juices with nitrogen and by irradiating at low temperatures, preferably at a frozen temperature. Under the conditions of oxygen exclusion and frozen temperatures, the antioxidant power of juices would be enhanced while the bleaching effect of irradiation would still be expected.

In summary, the results of this study indicated that irradiation at doses of pathogen inactivation had some beneficial effects, such as reductions of browning and increases in antioxidants. However, irradiation for the inactivation of some strains of *Salmonella* may increase MDA formation. The formation of MDA can be reduced by irradiating apple juice at frozen states.

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